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Prevalence, Incidence, and Spatial Dependence of Soybean mosaic virus in Iowa

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Abstract

The prevalence of soybean fields with plants infected with *Soybean mosaic virus* (SMV) in Iowa is assumed to be random, because the primary source of the virus is SMV-infected seed. Data collected from 2,500 soybean fields sampled over a 3-year period as part of the Iowa Soybean Disease Survey (2005 to 2007) were used to evaluate this assumption. SMV was first detected in early June of each year but counties in which it was first detected varied among years. Prevalence at the county scale at end of season was 32.3, 27.3, and 89.9% in 2005, 2006, and 2007, respectively. End-of-season incidence of SMV within SMV-positive counties was 1.5 to 25.0, 1.7 to 24, and 1.8 to 58% in 2005, 2006, and 2007, respectively. The number of fields in which plants infected with SMV were detected increased at the linear rate of approximately one new field every 2 days in 2007, compared with one new field every 22 days (2005) and 21 days (2006), with coefficients of determination (R^2) of 93.2 to 96.8% using the linear model. Weak spatial dependence for end-of-season SMV incidence was detected using Moran's Index, indicating that the risk for SMV incidence at the county scale within Iowa at the end of the growing season is not random.

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ABSTRACT

Lu, X., Robertson, A. E., Byamukama, E., and Nutter, F. W., Jr. 2010. Prevalence, incidence and spatial dependence of *Soybean mosaic virus* in Iowa. Phytopathology 100:931-940.

The prevalence of soybean fields with plants infected with *Soybean mosaic virus* (SMV) in Iowa is assumed to be random, because the primary source of the virus is SMV-infected seed. Data collected from 2,500 soybean fields sampled over a 3-year period as part of the Iowa Soybean Disease Survey (2005 to 2007) were used to evaluate this assumption. SMV was first detected in early June of each year but counties in which it was first detected varied among years. Prevalence at the county scale at end of season was 32.3, 27.3, and 89.9% in 2005, 2006, and 2007, respectively. End-of-season incidence of SMV within SMV-positive counties was 1.5 to 25.0, 1.7 to 24, and 1.8 to 58% in 2005, 2006, and 2007, respectively. The number of fields in which plants infected with SMV were detected increased at the linear rate of approximately one new field every 2 days in 2007, compared with one new field every 22 days (2005) and 21 days (2006), with coefficients of determination (R^2) of 93.2 to 96.8% using the linear model. Weak spatial dependence for end-of-season SMV incidence was detected using Moran's Index, indicating that the risk for SMV incidence at the county scale within Iowa at the end of the growing season is not random.

Soybean mosaic is a disease of soybean (*Glycine max* (L.) Merr.) caused by *Soybean mosaic virus* (SMV). This virus is found throughout the world wherever soybean crops are grown (9). Yield losses ranging from 8 to 35% (8,12,26,27,30) to as high as 86 to 94% have been documented (3,5). Yield components most affected are pod set, seed size, and seed weight (9). Furthermore, coinfection of soybean plants by SMV with other soybean viruses (e.g., *Bean pod mottle virus*) can have synergistic effects leading to yield losses greater than those caused by either virus alone (1,24,26).

Detection of SMV is best performed by directly testing for the presence of the virus rather than by assessing disease symptoms. Although most commercial soybean cultivars planted in Iowa are susceptible to SMV (9), soybean mosaic symptoms vary with soybean cultivar, virus strain, plant age at time of infection, and environment (6,9,14,15,28). Moreover, high temperatures that occur during the growing season in the Midwest can often mask SMV symptoms (10,15).

Seed from SMV-infected plants is considered to be the primary source of initial inoculum and for the long-distance dissemination of SMV into a soybean field (at planting) (13). In addition, 32 migratory aphid species belonging to 15 different genera, and the colonizing aphid, *Aphis glycines,* can acquire and transmit SMV to soybean plants in a nonpersistent manner (6,7,9,11).

An intensive statewide soybean disease survey (Iowa Soybean Disease Survey) was undertaken in Iowa during the 2005–07 growing seasons to determine the relative risks for all soybean diseases found in the state. These quantitative survey data, when coupled with global position systems (GPS) and geographic information systems (GIS) technologies, provided a unique opportunity to quantify and map spatial and temporal changes in the prevalence and incidence of SMV at the county and field scales over a 3-year period. In addition, the data allowed us to determine whether there is spatial dependence (clustering) for SMV at the county scale and, thus, test the hypothesis that SMV prevalence and incidence at the county scale is random in Iowa (because seed is the primary inoculum source). Therefore, the objectives of this study were to (i) quantify and map the prevalence and incidence of SMV in commercial soybean fields in Iowa and (ii) determine whether there is spatial dependence (clustering) for SMV at the county scale.

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MATERIALS AND METHODS

Sampling. A statewide soybean disease survey (Iowa Soybean Disease Survey) was carried out in Iowa over the course of three growing seasons, beginning in May 2005 and concluding in September 2007. Within each growing season, three to five soybean fields were sampled at growth stages V2-V3, R1-R2, R4-R5, and R6-R7 within each Iowa county by Iowa State University Extension Field Agronomists. Because the majority of soybean crops in Iowa are planted in mid-May, these growth stages were generally observed in beginning June, late June, beginning August, and late August. Thirty soybean plants from each soybean field were collected using a systematic sampling design (modified cross with 10 arms) (21). The length of individual arms within a field was proportional to the size of the field, with arm length of 20 to 60 m. In addition, soybean fields were also surveyed by the United States Department of Agriculture–National Agricultural Statistics Service (USDA-NASS) from July through August of each year. Again, 30 plants were collected from each soybean field surveyed by using a modified W systematic sampling design with three soybean plants being sampled at each of 10 sampling points. The geographical location (GPS coordinates) of each soybean field sampled and stage of soybean growth were recorded at the time each field was sampled.

Subsampling. The center leaflet from the topmost fully expanded trifoliate of each plant sampled was removed and the 30 leaflets (from each field) were divided into five, six-leaflet subsamples. Subsamples were labeled and stored in plastic bags at 4°C until sap extraction.

Sap extraction. Sap was extracted from each six-leaflet subsample using a leaf press (Ravenel Specialties Crop., Seneca, SC). The six leaflets from each subsample were placed between metal rollers and 4 to 5 ml of general extraction buffer (Agdia, Inc., Elkhart IN) was added as sap was being extracted from the rollers. Leaf sap from each subsample was collected into a 5-ml wax paper portion cup and then immediately dispensed into three 1.5-ml microcentrifuge tubes. Tubes were stored at –20°C until a single tube was thawed, and 100-µl aliquots were added to each well to test for the presence of SMV by enzyme-linked immunosorbent assay (ELISA) (22).

SMV detection. Sap samples were tested for the presence of SMV using a commercial double-antibody sandwich ELISA kit by following the recommended protocol (Agdia, Inc.). Incubation periods and temperatures for ELISA steps were overnight at 4°C for coating antibodies, overnight at 4°C for sap samples, 2 h at room temperature for enzyme-linked antibody conjugates, and 30 min for p-nitrophenylphosphate tablet hydrolysis. Absorbance was read at 405 nm using a Bio-tek EL 800 96-well plate reader (Winooski, VT). Each subsample and SMV-positive and -negative controls (Agdia, Inc.) were repeated in duplicate wells. A six-leaflet subsample was considered positive if the average absorbance value was equal to or greater than twice the value of the mean of the negative controls (30). Then, ≈5% of the subsamples were arbitrarily selected and retested to confirm the results.

SMV assessment. The prevalence (%) of SMV at the county (or field) scale was defined as number of counties (or fields) in which SMV was detected, divided by the total number of counties (or fields) tested × 100 (30). The incidence (%) of SMV at the field and county scales was defined as the number of subsamples testing positive for SMV, divided by the total number of subsamples tested from a field (or county) × 100 (30). Relationships between SMV prevalence and incidence at the end of each growing season were examined by plotting county prevalence data (X) with respect to mean SMV incidence data (Y) with corresponding counties (Sigma Plot 10; SPSS INC, Chicago). The proportion of the variation in SMV incidence at the county scale that was explained by SMV prevalence at the county scale (R^2) was determined by linear regression if the F statistic for each overall model (year) was $P \leq 0.05$ (Statistical Analysis System 9.01; SAS Institute, Cary, NC). In addition to F-statistic and coefficient of determination (R^2) values, model fit was further evaluated by determining the standard error of the estimate for y (SEE_y), where y is predicted SMV incidence, based on SMV prevalence and by visual inspection of residual plots (19, 30)

Temporal analysis. Cumulative SMV prevalence (%) and incidence (%) data were both plotted with respect to the day of year to illustrate pathogen (SMV) progress over time within each growing season. To choose the population growth model that best provided a linear relationship between cumulative prevalence (or cumulative incidence) with respect to time, the goodness-of-fit for each model was determined using the same model evaluation criteria describing above.

Spatial analysis. The prevalence and incidence of SMV at both the county and field scales were mapped using geographic information (GIS) systems software (ArcGIS; ESRI, Redlands, CA). In addition, the prevalence and incidence data in June, July, August, and September, as well as cumulative prevalence and cumulative incidence over each growing season, were mapped using ArcGIS to illustrate seasonal patterns in the temporal and spatial patterns of SMV process for each growing season. To test the hypothesis that the prevalence of SMV prevalence and incidence occur at random among Iowa counties, Moran's Index (Moran's I) analysis was used (17). Moran's I provides a measure of the global spatial autocorrelation of the overall clustering of data. Moran's I values range from –1 (indicating perfect dispersion) to +1 (indicating perfect correlation; that is, strong clustering). A zero value indicates a random spatial pattern. In this study, a positive value for this index indicates that nearby areas (county scale) have similar values for SMV prevalence (or incidence), indicating the presence of spatial autocorrelation (clustering). The prevalence data at the end of each month of the growing season were used to test for the presence of spatial autocorrelation among Iowa counties. The incidence data at the end of each month of the growing season were also used to test for the presence of spatial dependence among Iowa counties that had similar levels of SMV incidence.

RESULTS

Prevalence and incidence of SMV at the county scale. The prevalence of SMV for each individual month (June, July, August, and September) and cumulative prevalence by month were mapped at the county scale for all three growing seasons (2005, 2006, and 2007) (Figs. 1 to 3). Soybean plant samples collected by Iowa State University Extension Field Agronomists and NASS were combined.

SMV was first detected in plants collected from soybean fields on 7 June (day of year 158) in 2005 and 2006 and on 12 June (day of year 163) in 2007. The counties within which SMV-infected plants were first detected differed in each year of the survey (Figs. 1 to 3).

By the end of the 2005 growing season, SMV was present in 31 of 96 counties (32.3%) that were sampled and tested for SMV (Fig. 1). The incidence of SMV within SMV-positive counties was 1.5 to 25.0% (Fig. 4A). In this year, the largest increase in new SMV-positive counties occurred in August and September, with 10 and 11 new counties, respectively. The number of new SMV-positive counties in June and July was four and six, respectively. In 2006, 27 of all 99 counties (27.3%) tested positive for SMV (Fig. 2), and the incidence of SMV within SMV-positive counties was 1.7 to 24.0% (Fig. 4B). In 2007, the prevalence of SMV increased to 89 of 99 counties (89.9%) (Fig. 3), while the incidence of SMV within SMV-positive counties varied from 1.8 to as high as 58.0% (Fig. 4C). In both 2006 and 2007, the greatest change in new counties testing positive for SMV occurred in July, with 15 of 27 (55.6%) new counties testing positive in 2006 and 56 of 89 (62.9%) new counties in 2007 (Figs. 2 and 3). Cumulative prevalence at the county scale increased each month in all 3 years, the only exception being that no new counties were detected for SMV in September 2007.

SMV prevalence and incidence at the field scale. SMV was detected in 43 of 853 soybean fields (5.0%, SMV prevalence) and 103 of 4,265 subsamples (2.4%, SMV incidence) in 2005 (Table 1). In 2006, SMV was detected in 35 of 842 soybean fields (4.2%, prevalence) and 74 subsamples out of 4,210 (1.8%, incidence). In 2007, SMV prevalence and incidence increased approximately sevenfold over the previous 2 years, with 285 of 805 soybean fields (35.4%, prevalence) and 702 of 4,025 subsamples (17.4%, incidence) testing positive for SMV (Table 1).

Cumulative SMV prevalence at the field scale with respect to time (day-of-year) was similar for both 2005 and 2006. In contrast, cumulative SMV prevalence increased at a much faster rate in 2007 compared with 2005 and 2006. Cumulative SMV prevalence with respect to time was best fit by a linear model ($P < 0.0001$), with time explaining 93.2, 95.3, and 96.8% (R^2 values) of the variation in cumulative SMV prevalence for 2005, 2006, and 2007, respectively (Fig. 5A). Furthermore, the low SEE_y values (0.33, 0.29, and 2.29%, respectively) confirmed that the linear model was appropriate for the data. Slopes (change in virus prevalence with respect to time) were 0.045, 0.047, and 0.452 for 2005, 2006 and 2007, respectively. Thus, cumulative prevalence (disease risk) for fields with SMV-diseased plants in 2007 (0.452%) increased an order of magnitude more rapidly than in 2005 (0.045%) and 2006 (0.047%), despite similar dates of first detection of SMV (day of year 158 to 163) in all 3 years. Just as

the rate of change in virus incidence with respect to time within a field represents a measure of "disease risk", the rate of change of SMV prevalence over time at a much larger spatial scale also represents a measure of seasonal SMV disease risk (20).

There was a linear relationship between incidence of SMV and time at the field scale (Fig. 5B) for each year ($P < 0.0001$), with R^2 values of 94.6, 93.4, and 95.7%, respectively, for 2005, 2006, and 2007, respectively. The SEE_y values for the linear model were

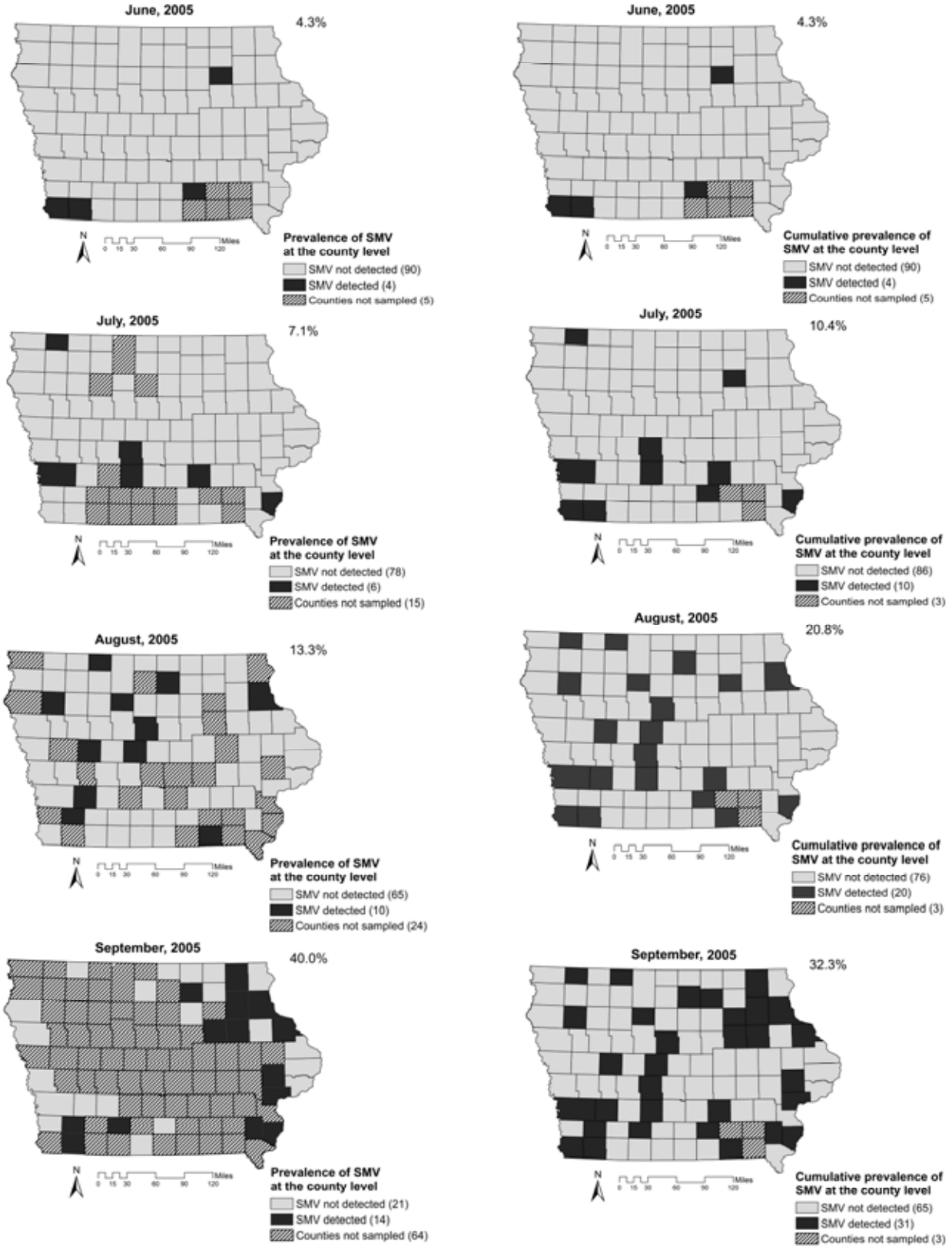

Fig. 1. Maps of Iowa indicating counties in which *Soybean mosaic virus* (SMV) either was not detected (light gray) or was detected (black), and counties which were not sampled (gray with diagonal lines). Maps in the left column show prevalence of SMV in each of the four sampling months, and the right column shows cumulative prevalence of SMV. The number at the upper right of each Iowa map is the percentage of sampled counties that were positive for SMV. Data were compiled from all samples collected and enzyme-linked immunosorbent assay tested in 2005.

0.16, 0.14, and 1.35%, respectively, and the slopes (change in SMV incidence with respect to time) were 0.024, 0.019, and 0.230% per day for 2005, 2006, and 2007, respectively. Thus, rates of SMV cumulative incidence were similar for 2005 and 2006 but the rate of cumulative SMV incidence in 2007 was 9.6 and 12.1 times greater than in 2005 and 2006, respectively.

Relationships between SMV prevalence and incidence. There was a significant linear relationship between SMV final

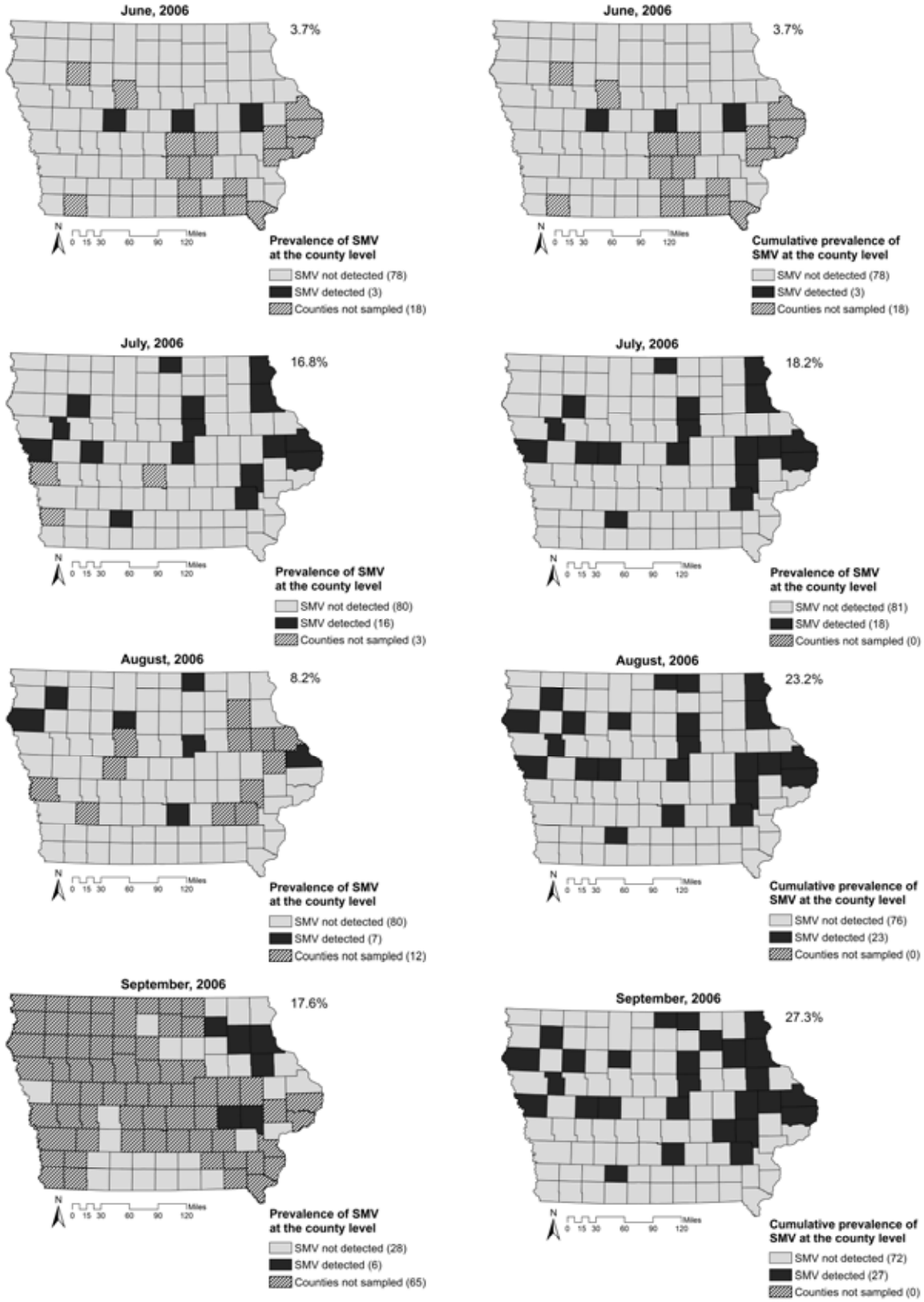

Fig. 2. Maps of Iowa indicating counties in which *Soybean mosaic virus* (SMV) either was not detected (light gray) or was detected (black), and counties which were not sampled (gray with diagonal lines). Maps in the left column show prevalence of SMV in each of the four sampling months, and the right column shows cumulative prevalence of SMV. The number at the upper right of each Iowa map is the percentage of sampled counties that were positive for SMV. Data were compiled from all samples collected and enzyme-linked immunosorbent assay tested in 2006.

prevalence (X) and SMV final incidence (Y) at the county scale for all three growing seasons (Fig. 6). In 2005, SMV prevalence explained (R^2) 72.4% of the variation in SMV incidence ($P < 0.0001$) (Fig. 6A). In 2006, 47.5% (R^2) of the variation in SMV incidence was explained by SMV prevalence ($P < 0.0001$) (Fig. 6B). In 2007, SMV prevalence explained 73.8% (R^2) of the variation in SMV incidence at the county scale ($P < 0.0001$) (Fig. 6C). For all 3 years of the disease survey, the higher the percentage of

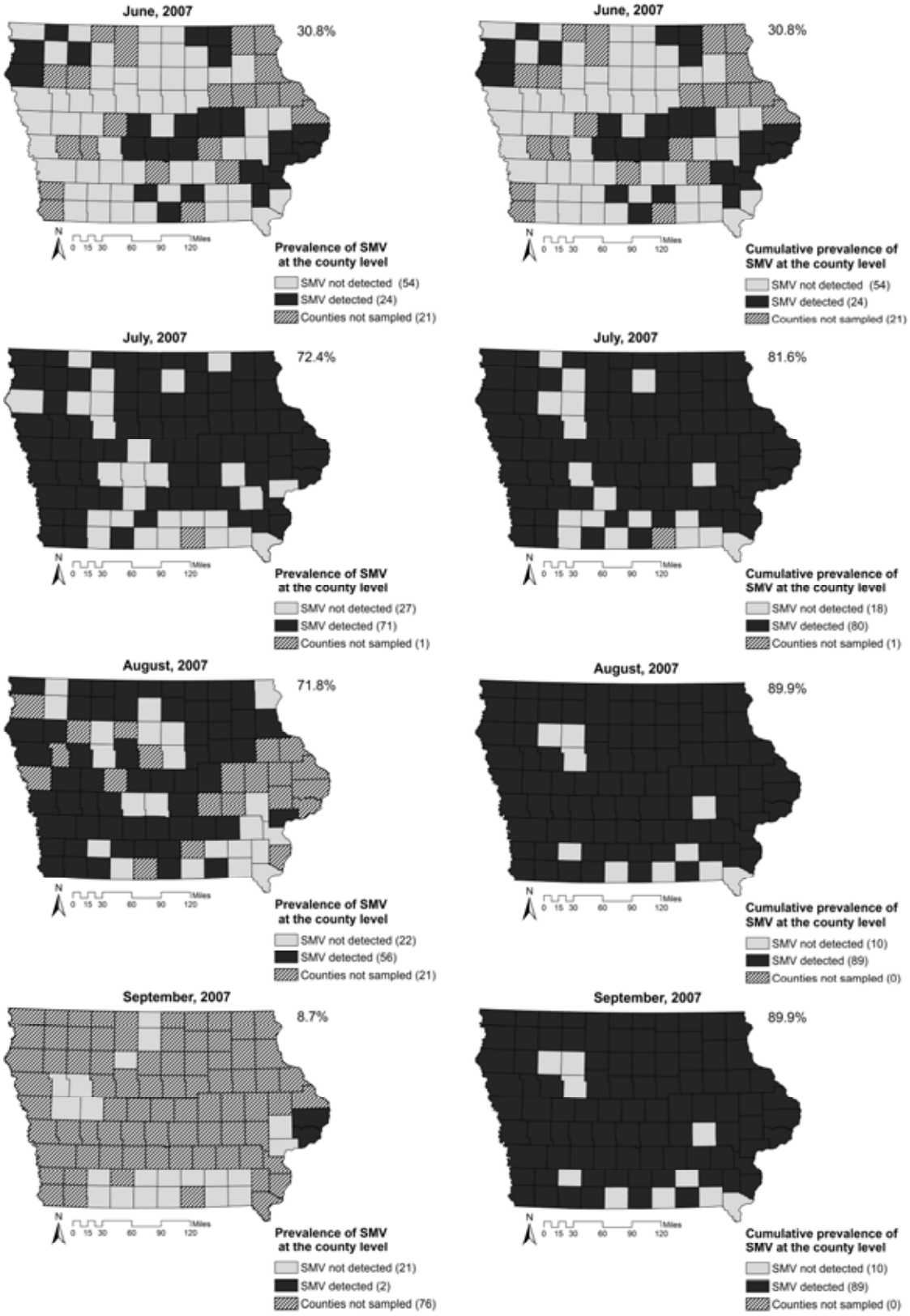

Fig. 3. Maps of Iowa indicating counties in which *Soybean mosaic virus* (SMV) either was not detected (light gray) or was detected (black), and counties which were not sampled (gray with diagonal lines). Maps in the left column show prevalence of SMV in each of the four sampling months, and the right column shows cumulative prevalence of SMV. The number at the upper right of each Iowa map is the percentage of sampled counties that were positive for SMV. Data were compiled from all samples collected and enzyme-linked immunosorbent assay tested in 2007.

fields with SMV in a county, the higher the mean incidence of SMV within that county.

Spatial dependence. No spatial dependence of SMV prevalence among counties for June, July, and August of the 2005 growing season was detected ($P > 0.1$), indicating a random pattern of counties testing positive for SMV in each month (Table 2). In September, however, significant (but weak) spatial dependence was detected (Moran's I 0.27, $P \leq 0.01$). Therefore, new

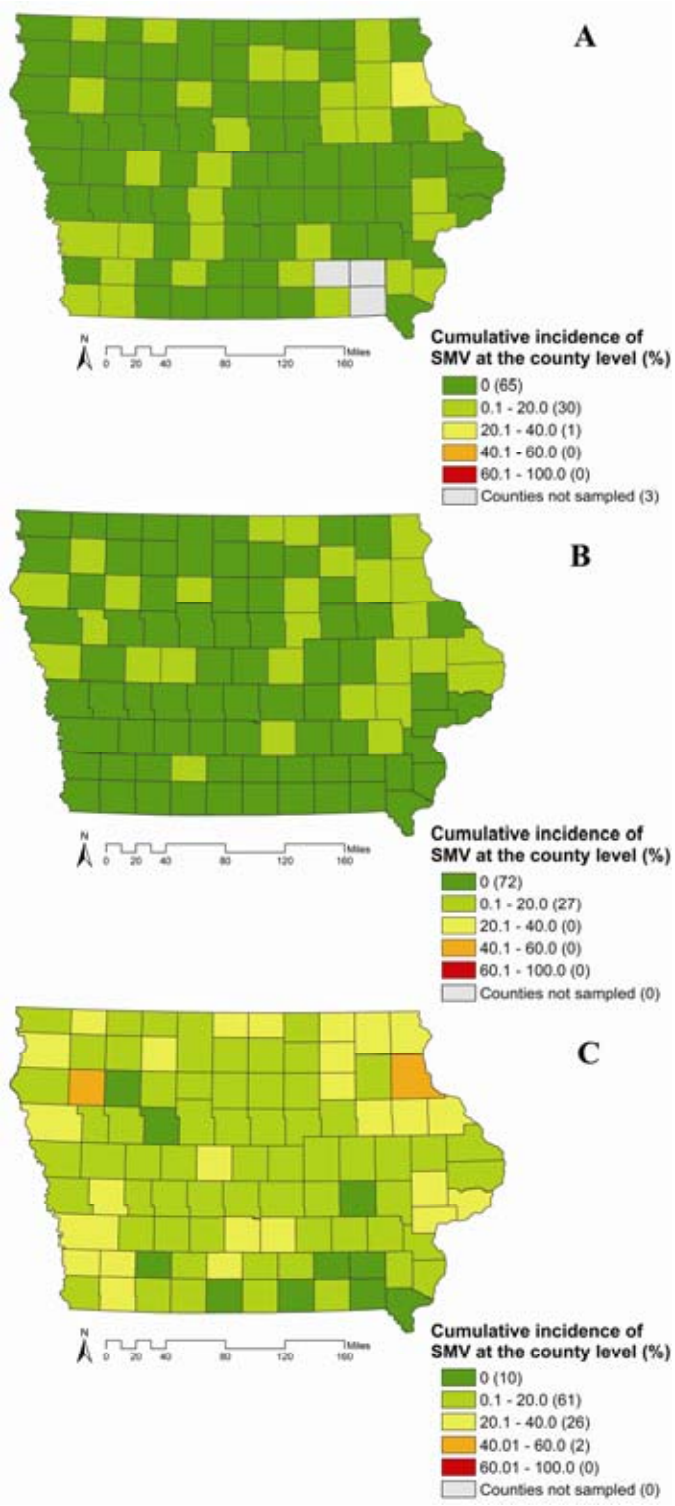

Fig. 4. Maps of Iowa showing the end-of-season cumulative incidence (%) of *Soybean mosaic virus* (SMV) in Iowa counties in **A,** 2005; **B,** 2006; and **C,** 2007. Colors for counties indicate incidence of SMV as follows: Dark green, 0%; light green, 0.1 to 20.0%; yellow, 20.1 to 40.0%; orange, 40.1 to 60%; and red, >60.1%. Counties that were not sampled are colored gray.

TABLE 1. End of season prevalence (%) and incidence (%) of *Soybean mosaic virus* (SMV) at the field scale as determined from the Iowa Soybean Disease Survey conducted in 2005–07

Year, sample source[a]	No. of soybean fields tested	Mean prevalence (%)	Mean incidence (%)
2005			
ISUE	715	5.7	2.7
NASS	138	1.4	0.4
Combined	853	5.0	2.4
2006			
ISUE	684	4.1	2.1
NASS	158	4.4	1.3
Combined	842	4.2	1.8
2007			
ISUE	624	36.5	19.0
NASS	181	31.5	13.7
Combined	805	35.4	17.4

[a] Iowa State University Extension (ISUE) Field Agronomists collected 30 soybean plants from each of three to five soybean fields per county at four different soybean growth stages (V2-V3, R1-R2, R4-R5, and R6-R7) to test for the presence (prevalence) and incidence of SMV in Iowa for each growing season. The United States Department of Agriculture National Agricultural Statistics Service (NASS) branch in Des Moines, IA, collected 30 soybean plants from each of 100 to 200 randomly selected soybean fields from late July to mid-August each growing season to test for the presence (prevalence) and incidence of SMV in Iowa.

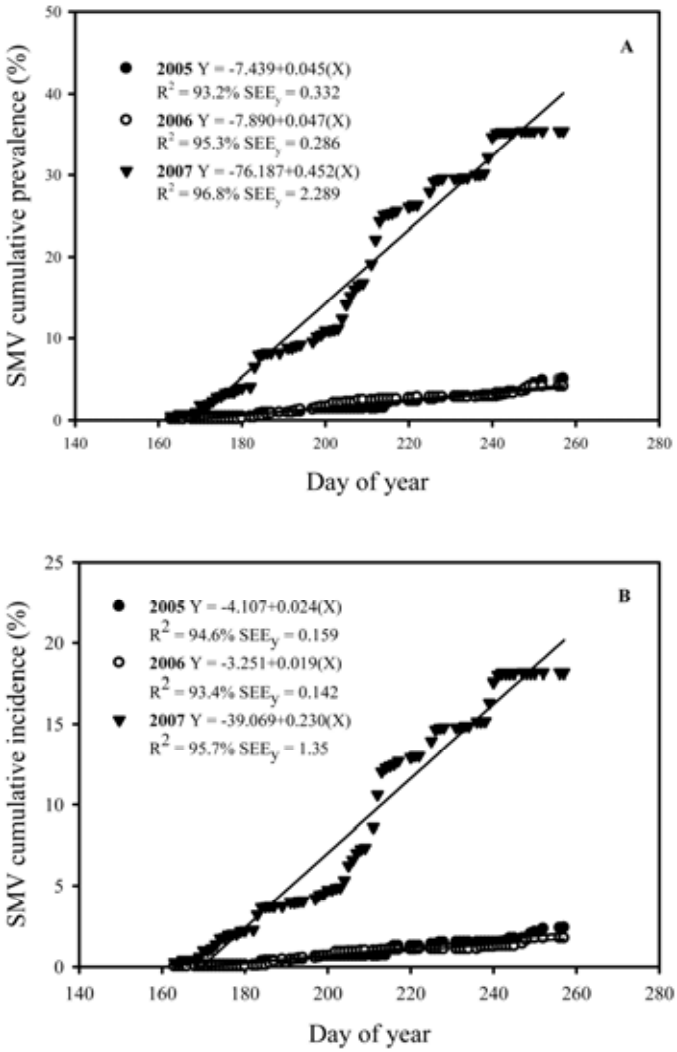

Fig. 5. Pathogen (*Soybean mosaic virus* [SMV]) progress curves depicting **A,** cumulative prevalence of SMV with respect to day of year in 2005, 2006, and 2007 and **B,** cumulative incidence of SMV with respect to day of year in 2005, 2006, and 2007.

counties within which SMV was detected in September 2005 were neighbored by Iowa counties with similar levels of SMV prevalence. Spatial dependence for counties testing positive for SMV at the county scale was not detected in any month of the growing season in 2006 ($P > 0.1$). In 2007, very weak spatial dependence was detected in June (Moran's I 0.13, $P < 0.05$) but no spatial dependence was detected in July, August, and September ($P > 0.1$) among counties testing positive for SMV prevalence, once more indicating a random pattern of counties testing positive for SMV (Table 2).

Based upon Moran's I, the spatial patterns of SMV incidence among counties soon after emergence (June) were random in 2005, 2006, and 2007 (Table 2). For all 3 years, however, the incidence of SMV within counties by the end of each growing season was clustered ($P < 0.01$), albeit the spatial dependence of SMV incidence was very weak in all 3 years (Table 2). Therefore, counties within which high incidences of SMV-infected plants were detected with neighbored tended to be each other (Fig. 4).

DISCUSSION

The Iowa Soybean Disease Survey was one of the largest, in-depth disease surveys undertaken for any crop. One of the most important outputs from the survey were data documenting the relative ranking of all soybean diseases in Iowa based upon prevalence and incidence data obtained over the 3-year period. In terms of prevalence, SMV ranked 11th out of 21 soybean pathogens detected in 2005 and 12th in 2006. In 2007, however, SMV ranked fourth in prevalence (35.4%), behind only bacterial blight (54.1%), brown spot (42.6%), and Rhizoctonia root rot (35.5%) (A. E. Robertson, *unpublished data*). Thus, based on our survey, the risk of soybean mosaic was low in 2005 and 2006 but reached moderate levels in 2007.

The low prevalence and very low incidence of SMV in 2005 and 2006 resulted in nondetectable yield losses, because healthy plants within soybean fields compensated for any yield reductions caused by the low incidence levels of SMV-infected soybean plants (30). Conversely, the incidence of SMV in 2007 within counties was as high as 55%; however, SMV incidence increased mostly late in the season. Therefore, the effect of SMV on yield was likely to be minimal, even in 2007, because yield losses as a result of SMV infection are greatest when soybean plants are infected prior to flowering (15). Irwin et al. (14) earlier reported a similar finding, in that SMV caused little damage (yield loss) except when seed-to-seedling transmission rates and vector intensity values are high during the first 4 to 5 weeks after seedling emergence.

Our study provides data on the spatial and seasonal dynamics of SMV prevalence and incidence during the growing season in Iowa. This has important implications as to (i) where and when soybean fields should be sampled and tested for the presence of SMV, (ii) where to conduct SMV disease surveys, and (iii) when to sample and test (evaluate) breeding lines or cultivars for resistance to SMV. The prevalence of SMV in soybean fields was lowest in June, which may be due to low initial inoculum that originates primarily from SMV-infected seed (9), or low population densities of vectors that limit early-season spread of SMV. We suspect that the higher prevalence and incidence levels of SMV in July, and even higher levels in August, were a function of more and more soybean fields exceeding our SMV detection threshold (due to greater plant-to-plant spread) because more plants in each field tested positive for SMV as the seasons progressed. In our study, the theoretical detection threshold was ≈3.3% (1 infected plant out of 30). Thus, (i) the higher the initial level of SMV seed infection within a soybean field, (ii) the earlier SMV vectors are present within a field, and (iii) the higher the population of aphid vectors of SMV, the sooner soybean fields will reach or exceed the detection threshold for our survey.

Epidemiologically, SMV-infected seed is the primary source of initial inoculum to initiate SMV epidemics (14). Moreover, the initial spatial pattern (at crop emergence) of SMV-infected soybean plants would be random due to seed-to-seedling transmission (22,30). Prior to the present study, no information was available concerning the spatial pattern of SMV epidemics at higher spatial scales (e.g., county, state, or region). This study

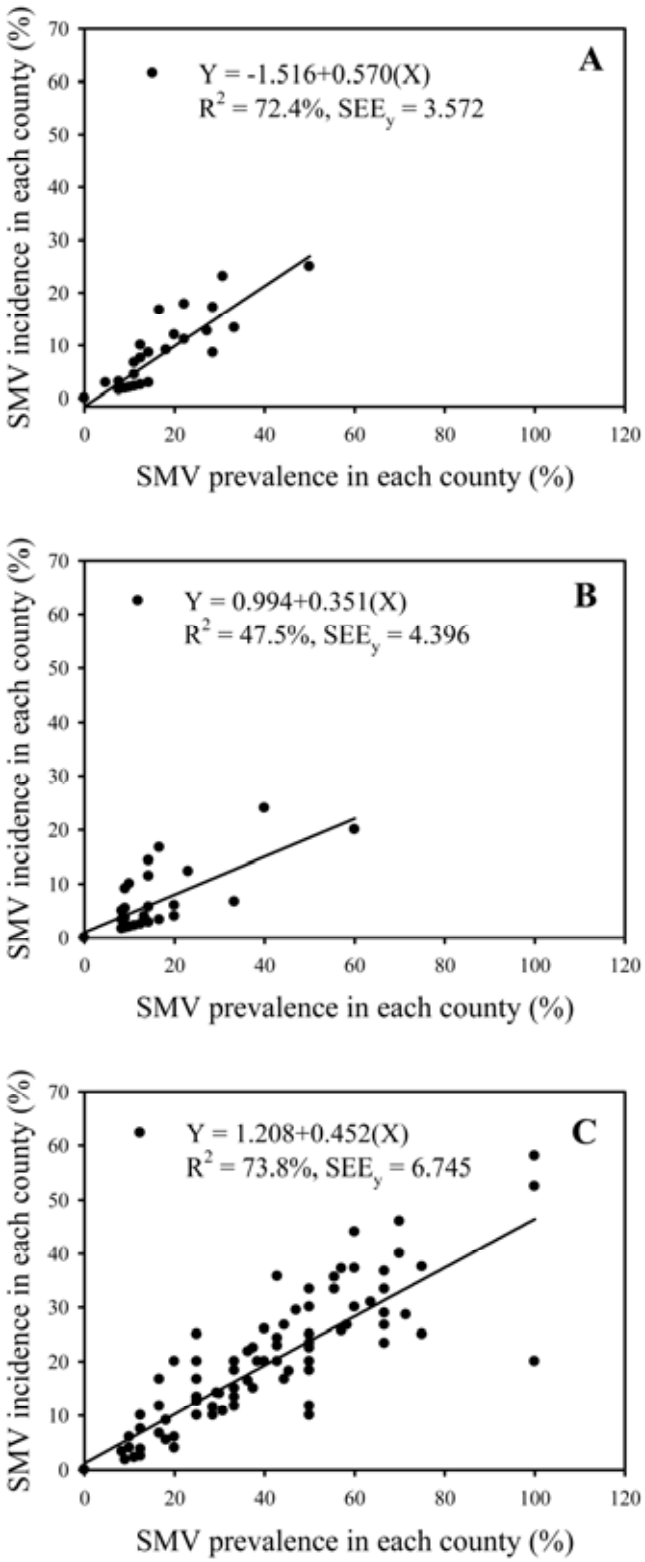

Fig. 6. Relationships between *Soybean mosaic virus* (SMV) prevalence (%) in each Iowa county (*X*) and corresponding SMV incidence (%) within that county (*Y*) in **A,** 2005; **B,** 2006; and **C,** 2007.

reveals that the seasonal and spatial risk for SMV within the state is not random. Based upon Moran's I analyses, there are months when counties with SMV can be expected to be neighbored by counties where SMV is also present. Conversely, counties where SMV was not detected can also be expected to be neighbored by counties that have similar levels of SMV prevalence. Counties with specific levels of SMV incidence also tend to be neighbored by other counties with similar levels of SMV incidence, again indicating that disease risk for SMV is not random among counties over time and space.

The significant ($P \leq 0.05$) spatial dependence that we detected for SMV incidence among counties from July to September in 2006 and 2007 may be due to two primary risk factors: (i) the spatial (local) distribution of SMV-infected seed lots or (ii) the presence of a neighborhood structure (i.e., clustering) among counties caused by spatial spread of SMV that was more favorable for some clusters of counties relative to other clusters of counties that had zero or low levels of SMV. In this second case, we propose that environmental factors affect the geographic differences in the rate of SMV spread (r). Although small seed companies are known to produce and sell their seed on a local scale (G. Munkvold, *personal communication*), we did not detect the presence of significant spatial dependence (clustering) of SMV prevalence or incidence among counties for the month of June in all three growing seasons. This strongly suggests that the processing and redistribution of SMV-infected soybean seed lots was not responsible for the weak spatial dependence that was detected after the month of June in all three growing seasons.

A neighbor structure for environmental factors favorable for SMV spread may be the cause of county clusters with similar levels of SMV. We hypothesize that this clustering is related to the propensity and spatial distribution of flights of noncolonizing aphid species that can acquire and transmit SMV in a nonpersistent manner (14,15). Previously, seasonal differences in the risk for SMV epidemics have been found to be related to year-to-year and geographical patterns of noncolonizing aphid species that increase the rate of SMV spread. If the incidence of SMV seed infection for soybean fields in our survey was related to the distribution of SMV-infected seed lots, and if the incidence of SMV-infected seed was >3.3% (based upon the detection threshold for our survey), then SMV would have been detected in June. However, if the incidence of SMV seed infection was <3.3%, then aphids would be required to acquire and spread SMV within these fields before SMV incidence would reach or exceed a detection threshold of 3.3%. Using published infection rates for plant-to-plant spread of SMV within soybean fields that range from slow (0.06 logits/day) to moderately fast (0.13 logits/day) (22), an initial SMV seed infection level of 0.1%, when coupled with the slowest reported infection rate for SMV epidemics (0.06 logits/day), would require ≈59 days after crop emergence (i.e., early August) to reach or exceed a detection threshold of 3.3% of the plants sampled (Fig. 7). If the fastest reported plant-to-plant infection rate is coupled with a 0.1% SMV incidence of seed infection, then soybean fields should reach or exceed the 3.3% detection threshold of our survey in just 28 days after crop emergence (July). Based on the curves in Figure 7, as the level of SMV seed infection approaches 3.3%, or the faster the rate of plant-to-plant spread in the field, the shorter the time it would take to reach the 3.3% detection threshold for our survey. The 2005 and 2006 growing seasons were not generally favorable for SMV temporal and spatial spread, and the likelihood (based on our survey) is that all three growing seasons in our survey had similar (low) levels of SMV seed infection; therefore, we propose that the higher levels of SMV prevalence and incidence detected in 2007 were due to the occurrence of environmental factors (such as higher vector populations) that differentially increased the rate

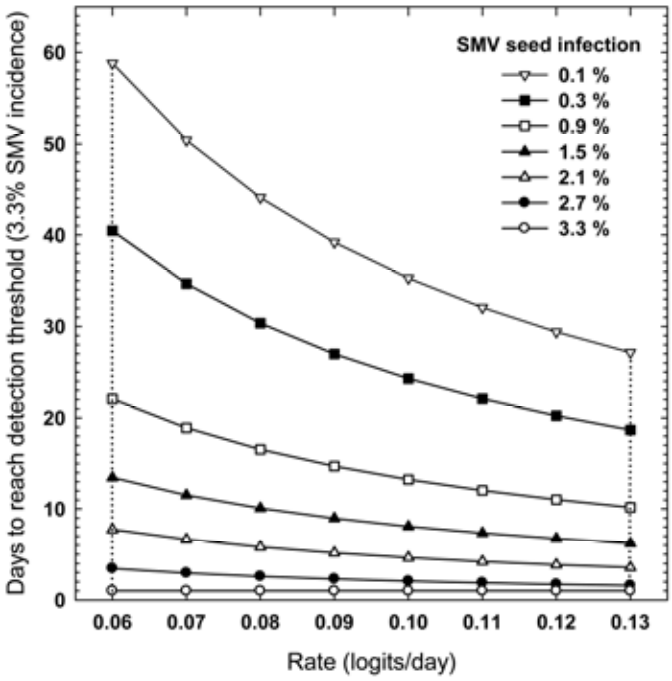

Fig. 7. Predicted time (days after crop emergence) to reach 3.3% detection threshold for *Soybean mosaic virus* (SMV) incidence within soybean fields as affected by the level of SMV seed infection and the rate of SMV infection by aphid vectors.

TABLE 2. Spatial analyses for prevalence and incidence *Soybean mosaic virus* (SMV) at the county scale in Iowa in 2005, 2006, and 2007 using Moran's Index (Moran's I)

	SMV prevalence			SMV incidence		
Year, month	Moran's I	*P* value	Pattern	Moran's I	*P* value	Pattern
2005						
June	0.09	0.08	Random	0.043	0.305	Random
July	0.05	0.33	Random	0.030	0.505	Random
August	–0.08	0.28	Random	–0.062	0.404	Random
September	0.27	0.01	Clustered	0.280	0.0001	Clustered
2006						
June	–0.05	0.50	Random	–0.049	0.480	Random
July	0.07	0.22	Random	0.204	0.0002	Clustered
August	–0.03	0.78	Random	0.162	0.005	Clustered
September	0.04	0.67	Random	0.210	0.0001	Clustered
2007						
June	0.13	0.04	Clustered	0.088	0.113	Random
July	0.08	0.15	Random	0.172	0.005	Clustered
August	0.05	0.33	Random	0.207	0.0001	Clustered
September	0.05	0.33	Random	0.250	0.0001	Clustered

of temporal spread within (and among) soybean fields and counties. Moreover, we propose that a neighborhood structure for the rate of SMV infection, rather than spatial clusters of similar SMV seed infection levels, was responsible for the spatial dependence (clustering) present among counties from July through September in 2005 and 2006 and all months in 2007.

The soybean aphid was detected in every county in each year of the Iowa Soybean Disease Survey (100% prevalence); however, soybean aphid population numbers for the apterous (colonizing) versus the alate (winged) forms of the soybean aphid were not determined in our survey. The risk of SMV prevalence and incidence at the county scale remained very low in 2005 and 2006, indicating that the mere presence or absence of the soybean aphid during these growing seasons had little association with SMV disease risk. This agrees with the results from a previous study that reported that the colonizing form of the soybean aphid had little impact on plant-to-plant spread of SMV in Iowa (23). Apparently, colonizing species, such as the soybean aphid, tend not to migrate to other plants when soybean aphid population densities are below a population density threshold and, therefore, plant-to-plant dissemination of SMV within soybean fields would have been limited. However, end-of-season prevalence and incidence of SMV at the field scale in soybean fields in the 2007 growing season was approximately seven times higher compared with previous growing seasons. The Pest Information Platform for Extension and Education (www.sbrusa.net) contains quantitative data concerning the population densities of soybean aphid in sentinel soybean plots that were collected during the growing season. During the 2006 growing season, reported aphid population densities in sentinel soybean plots in Iowa were zero to very low (0 to 5 aphids per plant) in northeast Iowa throughout the growing season. In 2007, however, soybean aphid population densities were considerably higher. From late July 2007 onward, soybean aphids were reported in all sentinel plot locations within the state, with threshold populations (>250 aphids per plant) (25) reported in northeast Iowa. Alate soybean aphid populations were also tracked from May through mid-October using a network of suction traps (www.ncipmc.org/traps). In 2005, the peak number of alate soybean aphids collected in four suction traps located in Iowa was ≈500 in 1 week (mid-July) whereas, in 2006, this number did not exceed 100 individual alates (25) throughout the growing season. In 2007, however, the peak number of alate soybean aphids collected per trap in 1 week was as high as 3,530, which occurred during late July to early August (www.ncipmc.org/traps). We hypothesize that the increased population densities of the soybean aphid in 2007 (particularly the alate form) had an impact on the significantly higher levels of both SMV prevalence and incidence in that year. It is well known that crowding of the adult apterae form when host plants are under stress can induce the reproduction of greater numbers of the alate form (16) which, in turn, will greatly increase alloinfection (31). Pathogen progress curves in 2007 (Fig. 4) for both SMV prevalence and incidence in soybean fields exhibited the fastest temporal increase from 20 July to 8 August (day of year 211 to 220), which coincides with the same time period when the alate populations were most abundant. These data suggest that alate soybean aphids may play a much greater role in the epidemiology and dissemination of SMV in soybean fields than previously thought. However, it should also be acknowledged that there are 32 known species of noncolonizing aphids that can also vector SMV (9) and, therefore, it cannot be assumed that the higher SMV prevalence and incidence levels in Iowa in 2007 were solely due to the alate form of the soybean aphid. Additional research is needed to better elucidate the role of alate soybean aphids versus the role of the 32 species of noncolonizing aphids in the dissemination of SMV within and among soybean fields.

We have shown a linear relationship between SMV prevalence (%) at the county scale with mean incidence (%) of SMV at the county scale. Thus, the more fields that tested positive for SMV within a county, the higher the average SMV incidence will be within that county. Although other studies have reported similar linear relationships between disease incidence and disease severity (2,18,29), this is the first study to show a quantitative relationship between the prevalence of a plant virus among fields and the incidence of a plant virus within fields at the county scale. This is important information concerning the allocation of limited resources when designing disease surveys. Based on our survey results, more emphasis can be placed on increasing the number of fields per county that are tested and less emphasis can be placed in the incidence of SMV within soybean fields, which required five composite ELISA tests per field in our study. For disease prevalence, a smaller number of bulked leaf samples could be tested by ELISA at a lower cost per field, and equations for group testing used to estimate the prevalence at the field scale (4).

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